

IN THE CLAIMS

Claims 1-62 (cancelled)

63. (new)A method for generating a specified amino acid other than methionine at the N-terminus of a target protein comprising:

expressing in a host cell, a nucleic acid encoding a fusion protein having an intein coding sequence adjacent to a coding sequence for the specified amino acid other than methionine in the target protein; and

cleaving the intein from the target protein so as to generate the specified N-terminal amino acid.

64. (new)A method according to claim 63 where the specified amino acid is cysteine or selenocysteine.

65. (new)A method for ligating a plurality of target proteins, the method comprising the steps of:

- (a) expressing from a first plasmid in a first host cell, a first fusion protein comprising a first target protein having a C-terminus fused to an intein or modification thereof;
- (b) expressing from a second plasmid in the first host cell or a second host cell, a second fusion protein comprising the second target protein having an N-terminus fused to an intein or modification thereof;
- (c) obtaining an extracellular preparation of the first fusion protein and an extracellular preparation of the second fusion protein;
- (d) adding a thiol reagent to the extracellular preparation of the first fusion protein whereby the first intein is cleaved so as to form a C-terminal thioester on the first target protein;

- (e) cleaving the second intein or modification thereof from the second target protein in the extracellular preparation of the second fusion protein and forming an N-terminal cysteine or selenocysteine on the second target protein; and
- (f) permitting ligation of the first target protein of step (d) with the second target protein of step (e).

66. (new)The method of claim 65, wherein the first intein is an Mth RIR1 intein (SEQ ID NO:24), wherein the Mth RIR1 is modified.

67. (new)The method of claim 65, wherein the second intein is an Mth RIR1 intein (SEQ ID NO:24), wherein the Mth RIR1 intein is modified.

68. (new)The method of claim 66 or 67, wherein the modification of the Mth RIR1 intein comprises Ala instead of Asn¹³⁴ at the C-terminus, or Ala or Ser instead of Cys¹ at the N-terminus.

69. (new)The method of claim 65, wherein the second target protein of step (e) is cleaved from the second intein in the presence of a thiol reagent or by modulating any of temperature, pH, salt, chaotropic agents or combinations thereof.

70. (new)The method of claim 65, wherein step (c) further comprises: purifying from the extracellular preparation, the first or second fusion protein.

71. (new)The method of claim 70, wherein the step of purifying the fusion protein further comprises binding to a chitin resin column.

72. (new)The method of claim 65, wherein the first and second plasmids are capable of expression in at least one cell type selected from the group consisting of a bacterial, yeast, plant, insect and mammalian cell type.

73. (new)A method for ligating a first and a second target protein comprising

(a) combining in a mixture

(i) a first target protein having a C-terminus , wherein the C-terminus comprises a thioester formed by cleavage of a first intein or modification thereof from a first fusion protein, the first fusion protein comprising the first intein or modification thereof positioned at the C-terminus of the first target protein; and

(ii) a second target protein having an N-terminus, wherein the N-terminus is a cysteine or selenocysteine amino acid, the N-terminus cysteine or selenocysteine resulting from induced cleavage of a second intein or modification thereof in a second fusion protein, the second fusion protein comprising the second intein or modification thereof positioned at the N-terminus of the second target protein;

wherein the first and second intein or modification thereof may be the same or different; and

(b) ligating the first and second target proteins.

74. (new)A method for cyclization of a target protein, the method comprising the steps of:

(a) expressing from a plasmid in a host cell, a target protein having a C-terminus and an N-terminus wherein a first intein or modification thereof is fused at the C-terminus, and a second intein or modification thereof is fused at the N-terminus adjacent to a

cysteine or selenocysteine amino acid on the target protein;

- (b) obtaining an extracellular preparation of the expressed fusion protein;
- (c) cleaving the fusion protein to remove the first and second inteins and obtaining the target protein having a C-terminal thioester and an N-terminal cysteine or selenocysteine; and
- (d) permitting intramolecular ligation of the N-terminus to the C-terminus of the target protein to form a cyclized protein.

75. (new)The method of claim 74, wherein the intein is an Mth RIR1 intein (SEQ ID NO:24), and the Mth RIR1 is modified wherein the modification comprises at the C-terminus, Ala instead of Asn¹³⁴ or at the N-terminus, Ala or Ser instead of Cys¹.

76. (new)The method of claim 74, wherein the N-terminal cysteine or selenocysteine is formed by cleavage of the intein or modification thereof from the target protein by modulating any of temperature, pH, salt, chaotropic agents or combinations thereof and the C-terminal thioester on the target protein is formed by adding a thiol reagent.

77. (new)The method of claim 74, wherein step (b) further comprises: purifying the fusion protein from the extracellular preparation.

78. (new)The method of claim 74, wherein the step of purifying the fusion protein further comprises binding to a chitin resin column.

79. (new) The method of claim 74, wherein the plasmid is capable of expression in at least one cell type selected from the group consisting of a bacterial, yeast, plant, insect and mammalian cell type.

80. (new) A method for cyclization of a target protein; comprising:
adding, to a fusion protein comprising (i) an intein or modification thereof at a C-terminus of the target protein and (ii) a cysteine or selenocysteine at an N-terminus of the target protein, a thiol reagent for cleaving the intein from the target protein so as to form a C-terminal thioester on the target protein; and

permitting intramolecular ligation of the C-terminal thioester to the N-terminal cysteine or selenocysteine for cyclization of the target protein.

81. (new) A cyclic protein produced by the method of claim 74.

82. (new) A method for polymerizing a plurality of target proteins of one type in a preparation, said method comprising the steps of:

- (a) expressing from a plurality of plasmids in an in vivo expression system, a plurality of fusion proteins, the fusion proteins each comprising a target protein having a C-terminus and an N-terminus, the target protein having a first intein or modification thereof fused to the C-terminus, and a second intein or modification thereof fused to the N-terminus, wherein the first intein or modification thereof is capable of being cleaved to produce a C-terminal thioester; and the second intein or modification thereof is capable of being cleaved to form an N-terminal cysteine or selenocysteine;
- (b) obtaining an extracellular preparation of the plurality of expressed fusion proteins;

- (c) adding a thiol reagent to the target protein for cleaving the first intein or modification thereof to produce the C-terminal thioester and inducing cleavage of the second intein or modification thereof to produce the N-terminal cysteine or selenocysteine; and
- (d) permitting intermolecular ligation between the C-terminal thioester on one target protein with the N-terminal cysteine or selenocysteine on a second target protein for forming a polymer from a plurality of target proteins.

83. (new) The method of claim 82, wherein the first intein is an Mth RIR1 intein (SEQ ID NO:24) wherein the Mth RIR1 is modified.

84. (new) The method of claim 82, wherein the second intein is an Mth RIR1 intein (SEQ ID NO:24), wherein the Mth RIR1 intein is modified.

85. (new) The method of claim 83 or 84, wherein the modification of the Mth RIR1 intein comprises Ala instead of Asn¹³⁴ at the C-terminus or Ala or Ser instead of Cys¹ at the N-terminus.

86. (new) The method of claim 82, wherein the second intein is cleaved from the target protein by modulating temperature, pH, salt or chaotropic agents or combinations thereof.

87. (new) The method of claim 82, wherein the plasmids are capable of expression in at least one cell type selected from the group consisting of a bacterial, yeast, plant, insect and mammalian cell type.

88. (new) A method for polymerizing a plurality of target proteins of one type; comprising:

adding a thiol reagent to a fusion protein, the fusion protein comprising a target protein having an N-terminus and a C-terminus, wherein the C-terminus is fused to a first intein or modification thereof and the N-terminus is fused to a second intein or modification thereof where the first and the second intein may be the same or different inteins;

forming a C-terminal thioester and an N-terminal cysteine or selenocysteine by cleaving the first and second inteins or modifications thereof; and

allowing intermolecular ligation of the C-terminal thioester of one target protein with the N-terminal cysteine or selenocysteine at the N-terminus of another target protein to form a polymer from the plurality of target proteins.

89. (new) A polymer produced by the method of claim 82.

90. (new) A modified Mth RIR1 intein, the Mth RIR1 comprise SEQ ID NO: 24 and the modification comprising Ala instead of Asn¹³⁴ at the C-terminus or Ala or Ser instead of Cys¹ at the N-terminus.